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EXAMINER	
MYERS, CARLA J	

ART UNIT	PAPER NUMBER
1634	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentdocket@choate.com

Office Action Summary	Application No.	Applicant(s)	
	10/577,341	BIANCHI ET AL.	
	Examiner	Art Unit	
	Carla Myers	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 October 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-17,19-48,51-93,96-111,114-131 and 133-137* is/are pending in the application.

4a) Of the above claim(s) *128-131 and 133-137* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-17,19-48,51-93,96-111 and 114-127* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. This action is in response to the reply of October 1, 2009. Applicant's arguments and amendments to the claims have been fully considered but are not persuasive to place all claims in condition for allowance. All rejections not reiterated herein are hereby withdrawn.

This action contains new grounds of rejection necessitated by Applicant's amendments to the claims and is made Final.

Claims 1-17, 19-48, 51-93, 96-111, 114-131 and 133-137 are pending.

Claims 1-17, 19-48, 51-93, 96-111, and 114-127 have been examined herein.

Claims 128-131 and 133-137 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on December 19, 2008.

2. The amendment to the claims filed on October 1, 2009 does not comply with the requirements of 37 CFR 1.121(c) because the claims are not properly marked with respect to amendments set forth therein and the correct status identifiers have not been used to identify each of the claims that have been amended. In particular, claims 4 and 5 have been amended so that they depend from claim 1, rather than claim 2. However, claims 4 and 5 are not accompanied by the status identifier of "currently amended" and the claims do not include a marking in which "1" has been lined through and "2" has been underlined. Amendments to the claims filed on or after July 30, 2003 must comply with 37 CFR 1.121(c) which states:

(c) *Claims*. Amendments to a claim must be made by rewriting the entire claim with all changes (*e.g.*, additions and deletions) as indicated in this subsection, except when the claim is being canceled. Each amendment document that includes a change to an existing claim, cancellation of an existing claim or addition of a new claim, must include a complete listing of all claims ever presented, including the text of all pending and withdrawn claims, in the application. The claim listing, including the text of the claims, in the amendment document will serve to replace all prior versions of the claims, in the application. In the claim listing, the status of every claim must be indicated after its claim number by using one of the following identifiers in a parenthetical expression: (Original), (Currently amended), (Canceled), (Withdrawn), (Previously presented), (New), and (Not entered).

(1) *Claim listing.* All of the claims presented in a claim listing shall be presented in ascending numerical order. Consecutive claims having the same status of "canceled" or "not entered" may be aggregated into one statement (*e.g.*, Claims 1–5 (canceled)). The claim listing shall commence on a separate sheet of the amendment document and the sheet(s) that contain the text of any part of the claims shall not contain any other part of the amendment.

(2) *When claim text with markings is required.* All claims being currently amended in an amendment paper shall be presented in the claim listing, indicate a status of "currently amended," and be submitted with markings to indicate the changes that have been made relative to the immediate prior version of the claims. The text of any added subject matter must be shown by underlining the added text. The text of any deleted matter must be shown by strike-through except that double brackets placed before and after the deleted characters may be used to show deletion of five or fewer consecutive characters. The text of any deleted subject matter must be shown by being placed within double brackets if strike-through cannot be easily perceived. Only claims having the status of "currently amended," or "withdrawn" if also being amended, shall include markings. If a withdrawn claim is currently amended, its status in the claim listing may be identified as "withdrawn—currently amended."

(3) *When claim text in clean version is required.* The text of all pending claims not being currently amended shall be presented in the claim listing in clean version, *i.e.*, without any markings in the presentation of text. The presentation of a clean version of any claim having the status of "original," "withdrawn" or "previously presented" will constitute an assertion that it has not been changed relative to the immediate prior version, except to omit markings that may have been present in the immediate prior version of the claims of the status of "withdrawn" or "previously presented." Any claim added by amendment must be indicated with the status of "new" and presented in clean version, *i.e.*, without any underlining.

(4) *When claim text shall not be presented; canceling a claim.*

(i) No claim text shall be presented for any claim in the claim listing with the status of "canceled" or "not entered."

(ii) Cancellation of a claim shall be effected by an instruction to cancel a particular claim number. Identifying the status of a claim in the claim listing as "canceled" will constitute an instruction to cancel the claim.

(5) *Reinstatement of previously canceled claim.* A claim which was previously canceled may be reinstated only by adding the claim as a "new" claim with a new claim number.

New grounds of rejection necessitated by Applicant's amendments to the claims:

Claim Rejections - 35 USC § 112 second paragraph

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 4 and 5 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 4 and 5 are indefinite over the recitation of "the remaining amniotic fluid" because this phrase lacks proper antecedent basis.

4. The following rejections were previously set forth in the Office action of April 1, 2009 and has been modified herein to address the amendments to the claims.

Claim Rejections - 35 USC § 102

5. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-3, 5, 6, 12-15, 19, 22, 25-26, 28-31, 34, 35 and 38 are rejected under 35 U.S.C. 102(b) as being anticipated by Bianchi (Clinical Chemistry. Oct. 2001. 47: 1867-1869; cited in the IDS of May 10, 2007).

Bianchi teaches a method of prenatal diagnosis comprising: i) providing a sample of amniotic fluid fetal DNA; ii) analyzing the amniotic fluid fetal DNA by hybridization to obtain fetal genomic information; and iii) based on the fetal genomic information, providing a prenatal diagnosis (page 1867, col. 2 and page 1868, col. 1). In particular, Bianchi teaches obtaining an amniotic fluid sample from pregnant woman at 16-20 weeks of gestation, isolating DNA present in the amniotic fluid sample, performing real-time quantitative PCR using primers and a dual-labeled fluorescent TaqMan probe, and determining the sex of the fetus, thereby providing a prenatal diagnosis. Bianchi teaches that amniotic fluid contains 100 to 200 fold more fetal DNA per millimeter as compared to maternal plasma. The method of Bianchi is considered to be one that includes analysis of fetal DNA from cell-free amniotic fluid by genomic hybridization since the method of Bianchi comprises the isolation of fetal genomic DNA from cell-free amniotic fluid, the amplification of the isolated fetal genomic DNA, and the hybridization of the amplified genomic DNA to a labeled probe.

Regarding claim 2, Bianchi teaches that the amniotic fluid fetal DNA is obtained by providing the amniotic fluid sample from a pregnant woman, removing cells from the sample by centrifugation, and treating the remaining amniotic fluid so as to extract cell-free DNA (page 1867, col. 2).

Regarding claim 3, the centrifugation step removes substantially all of the cells from the amniotic fluid so that the amniotic fluid fetal DNA consists essentially of cell-free fetal DNA.

Regarding claim 5, Bianchi teaches that the amniotic fluid samples are first frozen, stored under appropriate conditions, and thawed at 37C prior to vortexing and treating to extract DNA (page 1867, col. 2).

Regarding claim 6, Bianchi teaches that after thawing, the samples are centrifuges to remove any remaining cells (page 1867, col. 2).

Regarding claims 12-13 and 19, Bianchi teaches amplifying the amniotic fluid fetal DNA by PCR using primers (page 1868, col. 1).

Regarding claims 14-15, Bianchi teaches hybridizing the fluorescently labeled TaqMan probe to the amplified amniotic fluid fetal DNA to produce labeled amniotic fluid fetal DNA (page 1868, col. 1).

Regarding claim 22, Bianchi teaches that the prenatal diagnosis comprises determining the sex of the fetus (page 1868 and Table 1).

Regarding claims 25, 26, 28-30, 34, and 35, in the method of Bianchi, the amniotic fluid samples were collected in cases in which the fetus was suspected to have a chromosomal abnormality as indicated by an advanced maternal age, abnormal maternal serum screening results, or detection of a fetal sonographic abnormality. In two of the fetuses, the chromosomal abnormality was trisomy 21 associated with Down syndrome (page 1868, col. 1 and Table 1).

Regarding claims 31 and 38, since the amniotic fluid was obtained from subjects having high levels of biochemical markers in maternal serum, the fetuses were at risk of a disease or condition not detectable by G-banding analysis of metaphase CGH.

Response to Remarks:

In the reply filed October 1, 2009, Applicants traversed this rejection by stating that the claims have been amended to recite analyzing the amniotic fluid fetal DNA by "genomic hybridization" to obtain fetal genomic information. It is asserted that Bianchi does not teach using genomic hybridization.

This argument has been fully considered but is not persuasive. The specification and claims do not provide a definition for "genomic hybridization" which would distinguish the claimed method over that of Bianchi and there is no art recognized definition for this phrase which distinguishes the claimed method over that of Bianchi. As recited in the dependent claims, methods of "genomic hybridization" include the analysis of fetal DNA using a cDNA array (claim 8) and the analysis of fetal DNA by genomic hybridization using amniotic fetal DNA that has been amplified, particularly by PCR (claims 12 and 13). The claims do not require, for example, a step in which genomic DNA isolated from amniotic fluid is directly hybridized to a DNA probe, and particularly a genomic DNA probe. Rather, genomic hybridization is considered to include the method disclosed by Bianchi wherein fetal genomic DNA is isolated from cell-free amniotic fluid, the isolated fetal genomic DNA is amplified by PCR, and the amplified fetal genomic DNA is hybridized to a probe. Accordingly, it is maintained that Bianchi anticipates the claimed invention because the prenatal diagnosis method of

Bianchi includes a step of analyzing fetal DNA from cell-free amniotic fluid by "genomic hybridization" to obtain fetal genomic information.

6. Claims 1, 2, 4, 14-17, 19, 21-26, 28-31, 33, 34, 35 and 38 are rejected under 35 U.S.C. 102(b) as being anticipated by Lapierre et al (Prenatal Diagnosis. 2000. 20: 123-131; cited in the IDS of September 13, 2007).

This rejection is applied to the claims to the extent that the claims encompass methods which provide a sample of fetal DNA from cell-free amniotic fluid, wherein the sample comprises both fetal DNA that is cell free and DNA originating from cells in amniotic fluid. The rejection also applies to the claims to the extent the claims encompass methods in which the sample is obtained form amniotic fluid in which the cells have been lysed.

Lapierre teaches a method of prenatal diagnosis comprising: i) providing a sample of amniotic fluid fetal DNA; ii) analyzing the amniotic fluid fetal DNA by hybridization to obtain fetal genomic DNA; and iii) based on the fetal genomic information, providing a prenatal diagnosis (pages 124-125). In particular, Lapierre teaches obtaining an amniotic fluid sample from pregnant woman at 14-35 weeks of gestation, isolating DNA present in the amniotic fluid sample, labeling the DNA using Cyanine 3 (Cy3) and Fluor X-Amido using nick translation and subjecting the labeled DNA to comparative genomic hybridization (CGH) analysis using normal male metaphase chromosomal probes (page 124), and determining the karyotype of the fetus, as well as the sex of the fetus, thereby providing a prenatal diagnosis (pages 124-125 and Table 1).

Lapierre teaches that a first portion of the sample comprising amniotic cells is removed (i.e., removing cell populations from the sample) to obtain a remaining amniotic material and treating the remaining 5-10 ml of amniotic fluid to extract high molecular weight DNA for CGH analysis (page 124, col. 1). The remaining amniotic fluid thereby contained both amniotic cells (i.e., "some cells") and cell-free fetal DNA, as is encompassed by the claims. Further, in the method of Lapierre, the amniotic fluid is treated to lyse the cells prior to the isolation of the DNA. Thereby this step results in the production of a sample of fetal DNA from cell-free amniotic fluid (i.e., amniotic fluid in which the cells have been lysed.

Regarding claims 14-17 and 19, Lapierre teaches labeling the amniotic fluid fetal DNA by nick translation using the fluorescent label Cy-3 (page 124, col. 2) Bianchi teaches that the amniotic fluid samples are first frozen, stored under appropriate conditions, and thawed at 37C prior to vortexing and treating to extract DNA (page 1867, col. 2).

Regarding claims 21, 23, 24 , 28-30, 33-35, Lapierre teaches that the prenatal diagnosis comprises detecting trisomy 13, 18 or 21 (Table 1) and the presence of the XX or XY chromosomes.

Regarding claim 22, Lapierre teaches that the prenatal diagnosis comprises determining the sex of the fetus (page 1868 and Table 1).

Regarding claims 25, 26, 28-30, 33, 34, and 35, in the method of Lapierre, the amniotic fluid samples were collected in cases in which the fetus was suspected to have a chromosomal abnormality as indicated by an advanced maternal age, high

levels of biochemical markers in maternal serum, or detection of a fetal ultrasound abnormality. The chromosomal abnormalities included trisomy 21, associated with Down syndrome (page 124 and Table 1).

Regarding claims 31 and 38, since the amniotic fluid was obtained from subjects having high levels of biochemical markers in maternal serum, the fetuses were at risk of a disease or condition not detectable by G-banding analysis of metaphase CGH.

Response to Remarks:

In the response, Applicants assert that the claims have been amended to recite “fetal DNA from cell-free amniotic fluid." It is stated that Lapierre does not teach the analysis of fetal DNA from cell-free amniotic fluid.

This argument has been fully considered but is not persuasive. The claims appear to encompass methods in which a sample is provided that contains both fetal DNA from cell-free amniotic fluid that includes both cell-free DNA and DNA from cells present in the remaining amniotic fluid. This is evidenced by the fact that claim 4 which recites that the amniotic material comprises "some cells." The claims thereby do not require that the cell-free amniotic fluid does not contain any cells. Rather, the claims permit the amniotic fluid to contain some cells.

The method of Lapierre thereby anticipates the claimed invention because Lapierre teaches that a first portion of the sample comprising amniotic cells is removed (i.e., removing cell populations from the sample) to obtain a remaining amniotic material and treating the remaining 5-10 ml of amniotic fluid to extract high molecular weight DNA for CGH analysis (page 124, col. 1). The remaining amniotic fluid thereby

contained both amniotic cells (i.e., "some cells") and cell-free fetal DNA, as is encompassed by the claims. Further, in the method of Lapierre, the amniotic fluid is treated to lyse the cells prior to the isolation of the DNA. Thereby this step results in the production of a sample of fetal DNA from cell-free amniotic fluid (i.e., amniotic fluid in which the cells have been lysed.

Claim Rejections - 35 USC § 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 7-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bianchi (Clinical Chemistry. 2001. 47: 1867-1829) in view of Pinkel (U.S. Patent No. 6,210,878).

The teachings of Bianchi (2001) are presented above. Bianchi does not teach detecting the amniotic fluid fetal nucleic acids using a cDNA array or an oligonucleotide array.

However, Pinkel teaches methods for detecting genetic abnormalities, and particularly teaches the application of such methods to the analysis of amniotic fluid for prenatal diagnosis (col. 9, lines 48-49), wherein target nucleic acids are detected using an array of cDNA probes (col. 9, line 50-64). Such cDNA arrays are considered to be oligonucleotide arrays since the term oligonucleotide is not defined as being of a particular length or of particular structure/composition.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Bianchi so to have detected the fetal nucleic acids using a cDNA array as taught by Pinkel because this would have allowed for the simultaneous analysis of multiple fetal nucleic acids and would have provided an effective means for detecting target fetal nucleic acids as indicative of a prenatal diagnosis.

Response to Remarks:

In the reply filed October 1, 2009, Applicants traversed this rejection by stating Pinkel teaches a method of analyzing "amniotic fluid" but does not teach a method of analyzing cell-free amniotic fluid. This argument has been fully considered but is not persuasive because Pinkel was cited for teaching methods of prenatal diagnosis wherein target fetal nucleic acids are detected using an array of cDNA probes (col. 9, line 50-64). Further, Bianchi was cited for its clear teachings of methods of prenatal diagnosis by analyzing fetal DNA from cell-free amniotic fluid.

The response further asserts that Bianchi is not concerned with prenatal applications using DNA from cell-free amniotic fluid, but is instead interested in the biological origins of such DNA.

This argument has also been fully considered but is not persuasive. The method of Bianchi is in fact a method of prenatal diagnosis since Bianchi teaches analyzing fetal genomic DNA obtained from cell-free amniotic fluid to determine the sex of the fetus. Determining the sex of a fetus is considered to be a type of prenatal diagnosis. Further, the teachings of Bianchi are not limited to only the analysis of fetal DNA from cell-free

amniotic fluid to determine its biological origin. Rather, Bianchi clearly teaches that the cell-free fetal DNA in amniotic fluid is a valuable source of DNA for prenatal diagnosis and other "clinical applications." Bianchi discusses the fact that fetal DNA is used in a variety of clinical applications, including the "diagnosis of gender," Rhesus D genotype, single gene disorders, and aneuploidy (page 1867, col. 2). Bianchi also teaches that there is approximately 100 to 200 times more fetal DNA per milliliter of amniotic fluid as compared with maternal plasma and that “this higher amount may permit new studies and clinical applications to be performed on this typically discarded sample.” Accordingly, it is maintained that it would have been obvious to have modified the method of Bianchi so to have detected the fetal nucleic acids using a cDNA array as taught by Pinkel because this would have allowed for the simultaneous analysis of multiple fetal nucleic acids and would have provided an effective means for detecting target fetal nucleic acids as indicative of a prenatal diagnosis, particularly since Bianchi exemplifies using the fetal DNA in cell-free amniotic fluid to perform the prenatal diagnosis of determining the sex of the fetus and teaches that the very high concentration of fetal DNA in cell-free amniotic fluid permits the effective use of this DNA in clinical applications, such as prenatal diagnosis.

8. Claims 7, 9 and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bianchi (Clinical Chemistry. 2001. 47: 1867-1829) in view of Fan (Genome Research. 2000. 10:853-860).

The teachings of Bianchi (2001) are presented above. Bianchi does not teach detecting the amniotic fluid fetal nucleic acids using a SNP array or an oligonucleotide array.

However, Fan teaches methods for simultaneously screening for a large number of SNPs associated with genetic disorders using arrays having immobilized thereon oligonucleotide probes that are specific for SNPs. The high density tag arrays consist of over 64,000 probes (page 853) and provide for quantitative hybridization results, thereby permitting the determination of allele frequency in a DNA sample (abstract).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Bianchi so to have detected the fetal nucleic acids using the oligonucleotide SNP array taught by Fan in order to have provided a highly effective means for simultaneously screening the fetal DNA for the presence of SNPs associated with genetic diseases, thereby providing a rapid and sensitive method of prenatal diagnosis.

Response to Remarks:

In the reply filed October 1, 2009, Applicants traversed this rejection by stating Fan does not teach the application of their work to prenatal diagnosis. This argument is not persuasive because Fan was not cited for teaching a method of prenatal diagnosis, but rather was cited for its teachings of methods for simultaneously screening for a large number of SNPs associated with genetic disorders using arrays having immobilized thereon oligonucleotide probes that are specific for SNPs.

The response further asserts that Bianchi is not concerned with prenatal applications using DNA from cell-free amniotic fluid, but is instead interested in the biological origins of such DNA.

This argument was fully considered and addressed in paragraph 7 above and applies equally to the present grounds of rejection.

9. Claims 20, 36 and 37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bianchi (Clinical Chemistry. 2001. 47: 1867-1829) in view of Bianchi (U.S. Patent No. 5,714,325).

The teachings of Bianchi (2001) are presented above.

Regarding claim 20, Bianchi (2001) does not teach labeling the probe with a biotin label or a digoxigenin label.

However, Bianchi ('325) teaches methods of prenatal diagnosis wherein fetal nucleic acids are amplified by PCR and detected using a labeled probe (col. 14, line 13-line 26, and col. 15, lines 15-30). Bianchi also teaches labeling probes with biotin or digoxigenin (col. 18, lines 20-24, col. 20, line 62 to col. 21, line 11).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Bianchi (2001) so as to have labeled the nucleic acids with biotin or digoxigenin because this would have provided an equally effective means for labeling the nucleic acids to thereby facilitate their detection.

Regarding claims 36 and 37, Bianchi (2001) doesn't teach applying the prenatal diagnosis method to the detection of an X-linked disorder such as Duchenne muscular dystrophy.

However, Bianchi ('325) teaches performing prenatal diagnosis to detect chromosome X deletions associated with Duchenne muscular dystrophy using available probes (col. 14, lines 43-63).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the method of Bianchi (2001) to the detection of chromosome X deletions associated with Duchenne muscular dystrophy in order to have provided an effective method of prenatal diagnosis of Duchenne muscular dystrophy.

Response to Remarks:

In the reply filed October 1, 2009, Applicants traversed this rejection by stating that Bianchi (2001) is not concerned with prenatal applications using DNA from cell-free amniotic fluid. Applicants state that it would not be obvious to combine Bianchi (2001) with Bianchi ('325).

Applicant's argument that Bianchi (2001) is not concerned with prenatal applications was fully considered and addressed in paragraph 7 above and applies equally to the present grounds of rejection. Applicant's argument that it would not be obvious to combine the teachings of Bianchi (2001) with Bianchi ('325) are not persuasive because Applicant's do not provide any cogent arguments or evidence to support this assertion.

10. Claims 7, 11, 29, 32, 39-48, 51-54, 56, 59-67, 69-76, 79-88, 90-93, 96, 97, 99, 102, 103,104-111, 114, 115, 117, 118, 120, and 124-127 are rejected under 35 U.S.C.

103(a) as being unpatentable over Lapierre in view of Veltman et al (American Journal of Human Genetics. April 2002. 70: 1269-1276; cited in the IDS).

Note that this rejection applies to claims 7, 11, 29, 32, 39-48, 51-54, 56, 59-67, 69-76, 79-88, 90-93, 96, 97, 99, 102, and 103 to the extent that these claims encompass methods which provide a sample of fetal DNA from cell-free amniotic fluid, wherein the sample comprises both fetal DNA that is cell free and DNA originating from cells in amniotic fluid. The rejection also applies to the claims to the extent the claims encompass methods in which the sample is obtained form amniotic fluid in which the cells have been lysed.

Lapierre teaches a method of prenatal diagnosis comprising: i) providing a sample of amniotic fluid fetal DNA; ii) analyzing the amniotic fluid fetal DNA by hybridization to obtain fetal genomic DNA; and iii) based on the fetal genomic information, providing a prenatal diagnosis (pages 124-125). In particular, Lapierre teaches obtaining an amniotic fluid sample from pregnant woman at 14-35 weeks of gestation, isolating DNA present in the amniotic fluid sample, labeling the DNA using Cyanine 3 (Cy3) and Fluor X-Amido using nick translation and subjecting the labeled DNA to comparative genomic hybridization (CGH) analysis using normal male metaphase chromosomal probes (page 124), and determining the karyotype of the fetus, as well as the sex of the fetus, thereby providing a prenatal diagnosis (pages 124-125 and Table 1).

Lapierre teaches that a first portion of the sample comprising amniotic cells is removed (i.e., removing cell populations from the sample) to obtain a remaining

amniotic material and treating the remaining 5-10 ml of amniotic fluid to extract high molecular weight DNA for CGH analysis (page 124, col. 1). The remaining amniotic fluid thereby contained both amniotic cells and cell free fetal DNA. Accordingly, the method of Lapierre is one which analyzes amniotic fluid fetal DNA.

While Lapierre teaches analyzing amniotic fluid fetal DNA by CGH analysis to provide a prenatal diagnosis, Lapierre does not teach analyzing the amniotic fluid fetal DNA by array-based CGH.

However, Lapierre does teach that the new technology of microarray CGH provides a rapid and automated screening of chromosomal abnormalities commonly found during prenatal diagnosis including aneuploidies of chromosomes 13, 18, 21 and Y, deletions observed in Wolf Hirschhorn, Cri du chat syndromes and microdeletions as small as those observed in Prader-Willi and diGeorge syndromes (page 130, col.1). Lapierre suggests applying microarray CGH to isolated fetal DNA that has been amplified by PCR (page 130, col. 1).

Further, Veltman teaches the method of high-throughput array-based comparative genomic hybridization to detect chromosomal abnormalities. This method comprises providing a test sample having an unknown karyotype and labeled with a detectable agent; providing a reference sample of a known karyotype and labeled with a detectable agent; providing an array of genetic probes immobilized at discrete spots on an array and comprising a substantially complete third genome or a subset of a third genome; determining the binding of the test and reference samples to the genetic probes; and based on the relative binding pattern providing a diagnosis (page 1271 to

page 1272). Veltman states that telomeric chromosomal rearrangements may cause mental retardation, congenital anomalies, and miscarriage (abstract). It is stated that robustness and simplicity of array-based CGH make it highly suitable for introduction into the clinic as a rapid and sensitive automated diagnostic procedure (abstract and page 1271).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Lapierre so as to have performed the CGH analysis by array-based CGH in order to have achieved the advantages set forth by Lapierre and Veltman of providing a highly effective, rapid and automatable means for providing a prenatal diagnosis.

Regarding claims 29, 32, 39, 40, 42, 70, 73, 80-81, and 83, Lapierre suggests that microarray CGH analysis should be used to detect microdeletions such as those observed in Wolf Hirschhorn syndrome, Cri du chat syndrome, Prader-Willi syndrome and DiGeorge syndrome (page 130). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the microarray CGH method of Lapierre in view of Veltman to the detection of microdeletions in order to have provided a rapid and effective means for diagnosing the fetus for Wolf Hirschhorn syndrome, Cri du chat syndrome, Prader-Willi syndrome and DiGeorge syndrome.

Regarding claim 41, 82, and 107, Lapierre does not specifically teach applying the CGH analysis to prenatal diagnosis of a subtelomeric rearrangement. However, Veltman teaches that telomeric chromosomal rearrangements may cause mental

retardation, congenital anomalies and miscarriages (page 1269 and abstract). Veltman teaches applying array-based CGH to the detection of subtelomeric rearrangements using an entire subtelomeric clone set (page 1272 and Figure 1). It is stated that the array-based CGH subtelomeric assay is capable of screening all human subtelomeric regions in a single hybridization assay (page 1274, col. 2). Veltman concludes that array-based subtelomeric screening will have a profound impact on the diagnosis and genetic counseling of patients with mental retardation (page 1275).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the microarray CGH analysis of Lapierre to the detection of subtelomeric rearrangements in order to have provided an effective means for simultaneously screening for a multitude of subtelomeric rearrangements associated with mental retardation and congenital anomalies, thereby providing an effective method of prenatal diagnosis of these conditions.

Regarding claims 44-48, 90-93, and 109-111, in the array based CGH analysis of Veltman, the test and reference nucleic acids are each labeled with a fluorescent moiety of either Cy-3 or Cy-3 by random priming (page 1271, col. 2). Regarding claims 48, 93 and 111, while Veltman teaches that the test DNA is labeled with Cy3 and the reference DNA is labeled with Cy5, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified this methodology so as to have labeled with the test DNA with Cy5 and the reference DNA with Cy3 in order to have provided an equally effective means for labeling the DNAs.

Regarding claims 51-53, 96, and 114, in the array based CGH analysis of Veltman, high copy number repeats are suppressed by adding unlabeled human Cot-1 DNA to the test and reference sample prior to the contacting step (page 1271, col. 2).

Regarding claims 60, 103, and 126, Lapierre does not teach the methodology by which the karyotype of the genome of the reference nucleic acids was determined. However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have determined the karyotype of the reference genome by any art conventional technique, including the techniques of G-banding, metaphase CGH, FISH or SKY because each of these techniques would have provided an effective means for characterizing the karyotype as normal. Note that the claims do not require performing an active process step of determining the karyotype of the reference genome using the recited methods and since Lapierre and Veltman each teach that the reference genome is obtained from an individual with a normal karyotype, the methodology by which the karyotype was determined does not distinguish the claims over the prior art.

Regarding claims 61-62, 104-111, 114, 115, 117, 118, 120, and 124-127, in the array based CGH analysis of Veltman, the method further comprises measuring the intensity of the signals produced by the first and second detectable agents at the discrete spots on the array using a computer-assisted imaging system to obtain a fluorescent image of the array, and using the image analysis system to interpret the data and to display copy number ratios as a function of the genomic locus in the third genome (page 1271, col. 2 to page 1272, col. 1).

Regarding claim 63, Lapierre teaches that the prenatal diagnosis comprises determining the sex of the fetus (page 1868 and Table 1).

Regarding claims 64-65, 69-71, and 74 Lapierre teaches that the prenatal diagnosis comprises detecting trisomy 13, 18 or 21 (Table 1) and the presence of the XX or XY chromosomes.

Regarding claims 66, 67, 69-71, 74, 75, and 76, in the method of Lapierre, the amniotic fluid samples were collected in cases in which the fetus was suspected to have a chromosomal abnormality as indicated by an advanced maternal age, high levels of biochemical markers in maternal serum, or detection of a fetal ultrasound abnormality. The chromosomal abnormalities included trisomy 21, associated with Down syndrome (page 124 and Table 1).

Regarding claims 72 and 79, since the amniotic fluid was obtained from subjects having high levels of biochemical markers in maternal serum, the fetuses were at risk of a disease or condition not detectable by G-banding analysis of metaphase CGH.

Regarding claims 84-87, 90-93, 96 and 103, Lapierre teaches that the results of CGH analysis are compared to conventional cytogenetic analysis performed in situ by R-banding techniques (page 124, col. 1). Lapierre does not teach comparing the results of the CGH analysis of the test sample to a FISH analysis.

However, Veltman teaches comparing the results of array-based CGH to FISH analysis of a test sample to determine the consistency of the results, the sensitivity of detection and the selectivity of detection. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have further

modified the method of Lapierre so as to have compared the results of the array-based CGH analysis to results obtained using FISH in order to determine the consistency, sensitivity and selectivity of the detection method, in order to ensure that the accuracy of the prenatal diagnosis method.

Regarding claims 104-111, 114, 115, 117, 118, 120, 124-127, Lapierre teaches analyzing amniotic fluid fetal DNA samples from test subjects with ultrasound anomalies. Lapierre does not specifically teach analyzing amniotic fluid fetal DNA samples obtained from a fetus determined to have multiple congenital anomalies by sonographic examination, but having a normal karyotype, and particularly a normal karyotype which has been determined by a metaphase CGH analysis with a 550 band level resolution. However, Lapierre does teach that the method of array-based CGH analysis should be used to detect microdeletions in fetal nucleic acids as indicative of genetic disorders such as Prader-Willi and DiGeorge syndromes (page 130, col. 1). Further, Veltman teaches the use of array-based CGH to detect subtelomeric chromosomal rearrangements as indicative of disorders associated with mental retardation and genetic malformations (abstract and page 1269). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the array-based CGH analysis taught by Lapierre in view of Veltman to the analysis of sample nucleic acids obtained form test and control subjects that have the normal number of chromosomes and thereby a normal karyotype in order to have provided the advantage of generating a method of prenatal diagnosis that detected microdeletions and subtelomeric rearrangements associated with genetic disorders that

are not detectable by conventional karyotyping methods. Regarding claims 105 and 106, it is noted that the claims do not require an active process step of performing CGH analysis with a 550 band resolution. Further, it is noted that it is a property of the microdeletions and subtelomeric disclosed by Lapierre and Veltman that these chromosomal micro-abnormalities are not necessarily detectable by metaphase CGH analysis with a 550 band level resolution. At the time the invention was made, it was well known in the art that small chromosomal deletions or alterations could not be detected by CGH analysis of metaphase chromosomes. Accordingly, modification of the method of Lapierre so as to have detected the microdeletions or subtelomeric rearrangements by array-based CGH necessarily results in the detection of a chromosomal abnormality that is not detectable by metaphase CGH analysis with a 550 band level of resolution.

Regarding claim 127, Lapierre does not indicate the source of the reference nucleic acids and thereby does not teach that the test and reference nucleic acids are matched for fetal gender, site of sample acquisition, gestational age, and storage time. However, Lapierre does teach that the reference/control nucleic acids are obtained from karyotypically normal samples (page 123, col. 2). Lapierre also teaches performing CGH using both reference nucleic acids that are obtained from males and from females (Figure 2 and page 126). Further, the use of matched controls was well known in the art at the time the invention was made. Since the CGH method relies on detecting the occurrence of a genetic abnormality by comparing the results of a test sample with a reference sample, the ordinary artisan would have recognized the importance of

selecting matched test and reference samples for parameters which would effect the accuracy of the assay including fetal gender, means by which the amniotic fluid was obtained, gestation age and storage/handling of the amniotic fluid. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have performed the method of Lapierre using test and reference samples that were matched for fetal gender, site of sample acquisition, gestational age and storage time in order to have ensured that any differences observed between the test and reference samples were due to chromosomal changes, rather to non-related factors, thereby ensuring the accuracy of the prenatal diagnosis.

Response to Remarks:

In the reply, Applicants state that the claims have been amended to recite using fetal DNA from cell-free amniotic fluid. It is asserted that neither Lapierre nor Veltman teach analyzing fetal DNA from cell-free amniotic fluid.

This argument has been fully considered but is not persuasive because it is directed to limitations that are not recited in the claims.

Claims 104-111, 114, 115, 117, 118, 120, and 124-127 are not limited to a method which analyzes fetal DNA from cell-free amniotic fluid. Rather, the claims encompass the analysis of "amniotic fluid fetal DNA." In the method of Lapierre, a first portion of the sample comprising amniotic cells is removed (i.e., removing cell populations from the sample) to obtain a remaining amniotic material and the remaining 5-10 ml of amniotic fluid is treated to extract high molecular weight DNA for CGH analysis (page 124, col. 1). The remaining amniotic fluid thereby contained both

amniotic cells and cell free fetal DNA. Accordingly, the method of Lapierre is one which analyzes “amniotic fluid fetal DNA.”

Regarding claims 7, 11, 29, 32, 39-48, 51-54, 56, 59-67, 69-76, 79-88, 90-93, 96, 97, 99, 102, 103, these claims appear to encompass methods in which a sample is provided that contains both fetal DNA from cell-free amniotic fluid that includes both cell-free DNA and DNA from cells present in the remaining amniotic fluid. This is evidenced by the fact that claim 4 which recites that the amniotic material comprises "some cells." The claims thereby do not require that the cell-free amniotic fluid does not contain any cells. Rather, the claims permit the amniotic fluid to contain some cells. Lapierre teaches that a first portion of the sample comprising amniotic cells is removed (i.e., removing cell populations from the sample) to obtain a remaining amniotic material and treating the remaining 5-10 ml of amniotic fluid to extract high molecular weight DNA for CGH analysis (page 124, col. 1). The remaining amniotic fluid thereby contained both amniotic cells (i.e., “some cells”) and cell-free fetal DNA, as is encompassed by the claims. Further, in the method of Lapierre, the amniotic fluid is treated to lyse the cells prior to the isolation of the DNA. Thereby this step results in the production of a sample of fetal DNA from cell-free amniotic fluid (i.e., amniotic fluid in which the cells have been lysed.

11. Claims 7, 11, 29, 32, 39-48, 51-57, 59-67, 69-76, 79-88, 90-93, 96-100, 102-103, 116, 119, and 121 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lapierre in view of Veltman and further in view of Bianchi (Clinical Chemistry. Oct. 2001. 47: 1867-1869; cited in the IDS of May 10, 2007).

This rejection is applied to the claims to the extent that the claims encompass methods in which fetal DNA is from a sample of amniotic fluid in which substantially all of the cells have been removed from the amniotic fluid prior to the extraction of fetal DNA from the cell-free amniotic fluid.

The teachings of Lapierre and Veltman are presented above.

In particular, Lapierre teaches a method of prenatal diagnosis wherein the sample of amniotic fluid fetal DNA is obtained by removing a first portion of amniotic cells from an amniotic fluid sample to obtain a remaining amniotic material and treating the remaining 5-10 ml of amniotic fluid to extract high molecular weight DNA for CGH analysis (page 124, col. 1). The remaining amniotic fluid thereby contained both amniotic cells and cell free fetal DNA. Lapierre does not teach a method wherein the amniotic fluid sample is obtained by removing substantially all of the cells to obtain cell free DNA.

However, Bianchi teaches a method of prenatal diagnosis comprising obtaining an amniotic fluid sample from pregnant woman at 16-20 weeks of gestation, isolating DNA present in the amniotic fluid sample, performing real-time quantitative PCR using primers and a dual-labeled fluorescent TaqMan probe, and determining the sex of the fetus, thereby providing a prenatal diagnosis (page 1867, col. 2 and page 1868, col. 1). Bianchi teaches that the amniotic fluid fetal DNA is obtained by providing the amniotic fluid sample from a pregnant woman, removing cells from the sample by centrifugation, and treating the remaining amniotic fluid so as to extract cell-free DNA (page 1867, col. 2). The centrifugation step removes substantially all of the cells from the amniotic fluid

so that the amniotic fluid fetal DNA consists essentially of cell-free fetal DNA. Further, Bianchi teaches that the amniotic fluid samples are first frozen, stored under appropriate conditions, thawed at 37C prior, and remaining cells are removed by centrifugation prior to treatment to extract DNA (page 1867, col. 2). Bianchi teaches that amniotic fluid contains 100 to 200 fold more fetal DNA per millimeter as compared to maternal plasma (page 1868, col. 1). Bianchi states that "This higher amount may permit new studies and clinical applications to be performed on this typically discarded material" (page 1868, col. 1).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Lapierre so as to have treated the amniotic fluid as taught by Bianchi and to thereby have performed the prenatal diagnosis method using cell-free fetal DNA because Bianchi teaches that amniotic fluid comprises large quantities of cell-free fetal DNA and thereby this would have provided a highly effective source of fetal DNA for prenatal analysis and a convenient source of fetal DNA since the amniotic fluid supernatant is often available but discarded when the amniotic fluid has been obtained for alternative forms of analysis.

Regarding claims 39, 40, 42, 70, 73, 80-81, and 83, Lapierre suggests that microarray CGH analysis should be used to detect microdeletions such as those observed in Wolf Hirschhorn syndrome, Cri du chat syndrome, Prader-Willi syndrome and DiGeorge syndrome (page 130). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the

microarray CGH method of Lapierre in view of Veltman to the detection of microdeletions in order to have provided a rapid and effective means for diagnosing the fetus for Wolf Hirschhorn syndrome, Cri du chat syndrome, Prader-Willi syndrome and DiGeorge syndrome.

Regarding claim 41, and 82, Lapierre does not specifically teach applying the CGH analysis to prenatal diagnosis of a subtelomeric rearrangement. However, Veltman teaches that telomeric chromosomal rearrangements may cause mental retardation, congenital anomalies and miscarriages (page 1269 and abstract). Veltman teaches applying array-based CGH to the detection of subtelomeric rearrangements using an entire subtelomeric clone set (page 1272 and Figure 1). It is stated that the array-based CGH subtelomeric assay is capable of screening all human subtelomeric regions in a single hybridization assay (page 1274, col. 2). Veltman concludes that array-based subtelomeric screening will have a profound impact on the diagnosis and genetic counseling of patients with mental retardation (page 1275).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the microarray CGH analysis of Lapierre to the detection of subtelomeric rearrangements in order to have provided an effective means for simultaneously screening for a multitude of subtelomeric rearrangements associated with mental retardation and congenital anomalies, thereby providing an effective method of prenatal diagnosis of these conditions.

Regarding claims 44-48, and 90-93, in the array based CGH analysis of Veltman, the test and reference nucleic acids are each labeled with a fluorescent moiety of either

Cy-3 or Cy-3 by random priming (page 1271, col. 2). Regarding claims 48 and 93 Veltman teaches that the test DNA is labeled with Cy3 and the reference DNA is labeled with Cy5. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified this methodology so as to have labeled with the test DNA with Cy5 and the reference DNA with Cy3 in order to have provided an equally effective means for labeling the DNAs.

Regarding claims 51-53, in the array based CGH analysis of Veltman, high copy number repeats are suppressed by adding unlabeled human Cot-1 DNA to the test and reference sample prior to the contacting step (page 1271, col. 2).

Regarding claims 60, and 103, Lapierre does not teach the methodology by which the karyotype of the genome of the reference nucleic acids was determined. However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have determined the karyotype of the reference genome by any art conventional technique, including the techniques of G-banding, metaphase CGH, FISH or SKY because each of these techniques would have provided an effective means for characterizing the karyotype as normal. Note that the claims do not require performing an active process step of determining the karyotype of the reference genome using the recited methods and since Lapierre and Veltman each teach that the reference genome is obtained from an individual with a normal karyotype, the methodology by which the karyotype was determined does not distinguish the claims over the prior art.

Regarding claims 61-62, in the array based CGH analysis of Veltman, the method further comprises measuring the intensity of the signals produced by the first and second detectable agents at the discrete spots on the array using a computer-assisted imaging system to obtain a fluorescent image of the array, and using the image analysis system to interpret the data and to display copy number ratios as a function of the genomic locus in the third genome (page 1271, col. 2 to page 1272, col. 1).

Regarding claim 63, Lapierre teaches that the prenatal diagnosis comprises determining the sex of the fetus (page 1868 and Table 1).

Regarding claims 64-65, 69-71, and 74 Lapierre teaches that the prenatal diagnosis comprises detecting trisomy 13, 18 or 21 (Table 1) and the presence of the XX or XY chromosomes.

Regarding claims 66, 67, 69-71, 74, 75, and 76, in the method of Lapierre, the amniotic fluid samples were collected in cases in which the fetus was suspected to have a chromosomal abnormality as indicated by an advanced maternal age, high levels of biochemical markers in maternal serum, or detection of a fetal ultrasound abnormality. The chromosomal abnormalities included trisomy 21, associated with Down syndrome (page 124 and Table 1).

Regarding claims 72 and 79, since the amniotic fluid was obtained from subjects having high levels of biochemical markers in maternal serum, the fetuses were at risk of a disease or condition not detectable by G-banding analysis of metaphase CGH.

Regarding claims 84-87, 90-93, 96 and 103, Lapierre teaches that the results of CGH analysis are compared to conventional cytogenetic analysis performed in situ by

R-banding techniques (page 124, col. 1). Lapierre does not teach comparing the results of the CGH analysis of the test sample to a FISH analysis.

However, Veltman teaches comparing the results of array-based CGH to FISH analysis of a test sample to determine the consistency of the results, the sensitivity of detection and the selectivity of detection. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have further modified the method of Lapierre so as to have compared the results of the array-based CGH analysis to results obtained using FISH in order to determine the consistency, sensitivity and selectivity of the detection method, in order to ensure that the accuracy of the prenatal diagnosis method.

Response to Remarks:

In the reply, Applicants state that the claims have been amended to recite using fetal DNA from cell-free amniotic fluid. It is stated that Veltman does not teach the analysis of amniotic fluid and Lapierre does not teach the analysis of fetal DNA from cell-free amniotic fluid. It is further stated that Bianchi is not concerned with prenatal diagnosis and it would not be obvious to combine Bianchi with other references in the context of prenatal diagnosis.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Regarding Applicant's assertion that Bianchi is not concerned with methods of prenatal diagnosis, this argument is not persuasive because it does not take into consideration the complete teachings of the Bianchi reference. The method of Bianchi is in fact considered to be a method of prenatal diagnosis since Bianchi teaches analyzing fetal genomic DNA obtained from cell-free amniotic fluid to determine the sex of the fetus. Determining the sex of a fetus is considered to be a type of prenatal diagnosis. Further, Bianchi teaches that the cell-free fetal DNA in amniotic fluid is a valuable source of DNA for prenatal diagnosis and other "clinical applications." Bianchi discusses the fact that fetal DNA is used in a variety of clinical applications, including the "diagnosis of gender," Rhesus D genotype, single gene disorders, and aneuploidy (page 1867, col. 2). Bianchi also teaches that there is approximately 100 to 200 times more fetal DNA per milliliter of amniotic fluid as compared with maternal plasma and that "this higher amount may permit new studies and clinical applications to be performed on this typically discarded sample." Accordingly, it is maintained that it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Lapierre so as to have treated the amniotic fluid as taught by Bianchi and to thereby have performed the prenatal diagnosis method using cell-free fetal DNA because Bianchi teaches that amniotic fluid comprises large quantities of cell-free fetal DNA and thereby this would have provided a highly effective source of fetal DNA for prenatal analysis and a convenient source of fetal DNA since the amniotic fluid supernatant is often available but discarded when the amniotic fluid has been obtained for alternative forms of analysis.

12. Claims 58, 101, 122 and 123 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lapierre in view of Veltman and Bianchi, and further in view of Shah (U.S. Patent No. 6,916,621).

The teachings of Lapierre, Veltman and Bianchi are presented above.

Lapierre teaches using random priming to label the test and reference nucleic acid samples (page 1271, col. 2), but does not teach amplifying the test and reference (i.e., control) nucleic acids by PCR.

However, Bianchi teaches amplifying the amniotic fluid fetal DNA by PCR using primers prior to performing probe hybridization (page 1868, col. 1). Additionally, Veltman teaches amplifying the cloned DNA used as probes by degenerate oligonucleotide primed-PCR (DOP-PCR; page 1272, col. 1).

Shah (col. 15, lines 10-42) teaches methods of array-based CGH analysis wherein the sample (i.e., test and reference/control) nucleic acids are amplified by PCR. Shah teaches that degenerate primers can be used to amplify the sample nucleic acids in order to incorporate a labeled nucleotide into the nucleic acids. It is stated that PCR can be used in place of random primer extension to label nucleic acids (col. 15, lines 10-16).

In view of the teachings of Shah that it was conventional in the art to use PCR in place of random priming to label nucleic acids, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Lapierre so as to have amplified the test and reference (control) nucleic acids by PCR prior to performing the array-based CGH analysis in order to have provided an

alternative means for incorporating the label into the test and reference (control) nucleic acids to achieve the benefit of providing labeled target and reference nucleic acids to thereby facilitate their detection. Moreover, in view of the teachings of Veltman of using DOP-PCR to increase the quantity of nucleic acids, the teachings of Shah to amplify the test and control/reference nucleic acids and the teachings of Bianchi to use PCR to specifically increase the quantity of particular target nucleic acids in amniotic fluid, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Lapierre so as to have amplified the test and reference (control) nucleic acids by PCR prior to performing the array-based CGH analysis in order to have increased the quantity of these nucleic acids thereby improving the sensitivity of detection in the method of prenatal diagnosis.

Response to Remarks:

In the reply, Applicants state that the claims have been amended to recite using fetal DNA from cell-free amniotic fluid. It is stated that the combination of Lapierre, Veltman and Bianchi do not render obvious the use of fetal DNA from cell free amniotic fluid. These arguments have been addressed in full above and apply equally to the present grounds of rejection.

It is argued that Shah does not cure the deficiencies of Lapierre, Veltman and Bianchi because Shah does not teach the use of fetal DNA from cell-free amniotic fluid. This argument is not persuasive because one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck &*

Co., 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Shah was not cited for its teachings of analyzing fetal DNA from cell-free amniotic fluid. Rather, Shah was cited for its teachings of methods of array-based CGH analysis wherein the sample (i.e., test and reference/control) nucleic acids are amplified by PCR, the use of degenerate primers to amplify the sample nucleic acids in order to incorporate a labeled nucleotide into the nucleic acids and the use of PCR in place of random primer extension to label nucleic acids.

13. Claim 27 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lapierre in view of Bianchi (2001) and further in view of Sammons et al (U.S. Patent No. 5,948,278).

Note that this rejection applies to claim 27 to the extent that the claim encompasses methods which provide a sample of fetal DNA from cell-free amniotic fluid, wherein the sample comprises both fetal DNA that is cell free and DNA originating from cells in amniotic fluid. The rejection also applies to the claims to the extent the claims encompass methods in which the sample is obtained form amniotic fluid in which the cells have been lysed.

The teachings of Lapierre are presented above. Lapierre teaches obtaining the amniotic fluid sample from woman of “advanced maternal age” but does not specifically teach that the woman are age 35 or older.

Sammons teaches that amniocentesis increases a woman’s risk of miscarriage and therefore amniocentesis is generally offered primarily to woman over the age of 35

since woman over the age of 35 have a statistically greater probability of bearing children with congenital defects (col. 1, lines 43-49).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have specifically obtained the amniotic fluid samples from pregnant woman over the age of 35 because these woman would be at a greater risk of having fetuses with a genetic abnormality, and thereby would benefit most from prenatal diagnosis.

Response to Remarks:

In the reply, Applicants state that the claims have been amended to recite using fetal DNA from cell-free amniotic fluid and Lapierre does snot teach using fetal DNA from cell-free amniotic fluid.

This argument is the same as that addressed in full above and applies equally to the present grounds of rejection.

14. Claims 68 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lapierre in view of Veltman and further in view of Sammons et al (U.S. Patent No. 5,948,278).

Note that this rejection applies to claim 68 to the extent that the claim encompasses methods which provide a sample of fetal DNA from cell-free amniotic fluid, wherein the sample comprises both fetal DNA that is cell free and DNA originating from cells in amniotic fluid. The rejection also applies to the claims to the extent the claims encompass methods in which the sample is obtained form amniotic fluid in which the cells have been lysed.

The teachings of Lapierre and Veltman are presented above. Lapierre teaches obtaining the amniotic fluid sample from woman of "advanced maternal age" but does not specifically teach that the woman are age 35 or older.

Sammons teaches that amniocentesis increases a woman's risk of miscarriage and therefore amniocentesis is generally offered primarily to woman over the age of 35 since woman over the age of 35 have a statistically greater probability of bearing children with congenital defects (col. 1, lines 43-49).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have specifically obtained the amniotic fluid samples from pregnant woman over the age of 35 because these woman would be at a greater risk of having fetuses with a genetic abnormality, and thereby would benefit most from prenatal diagnosis.

Response to Remarks:

In the reply, Applicants state that the claims have been amended to recite using fetal DNA from cell-free amniotic fluid and Lapierre does snot teach using fetal DNA from cell-free amniotic fluid.

This argument is the same as that addressed in full above and applies equally to the present grounds of rejection.

15. Claims 77 and 78 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lapierre in view of Veltman and further in view of Bianchi (U.S. Patent No. 5,714,325).

Note that this rejection applies to the claims to the extent that the claims encompass methods which provide a sample of fetal DNA from cell-free amniotic fluid,

wherein the sample comprises both fetal DNA that is cell free and DNA originating from cells in amniotic fluid. The rejection also applies to the claims to the extent the claims encompass methods in which the sample is obtained form amniotic fluid in which the cells have been lysed.

The teachings of Lapierre and Veltman are presented above. The combined references do not teach applying the prenatal diagnosis method to the detection of an X-linked disorder such as Duchenne muscular dystrophy.

However, Bianchi ('325) teaches performing prenatal diagnosis to detect chromosome X deletions associated with Duchenne muscular dystrophy using available probes (col. 14, lines 43-63).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the method of Lapierre in vie w of Veltman to the detection of chromosome X deletions associated with Duchenne muscular dystrophy in order to have provided an effective method of prenatal diagnosis of Duchenne muscular dystrophy.

Response to Remarks:

In the reply, Applicants state that the claims have been amended to recite using fetal DNA from cell-free amniotic fluid and Lapierre does snot teach using fetal DNA from cell-free amniotic fluid.

This argument is the same as that addressed in full above and applies equally to the present grounds of rejection.

16. Claim 89 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lapierre in view of Veltman and further in view of Pinkel (U.S. Patent No. 5,665,549; cited in the IDS).

Note that this rejection applies to claim 89 to the extent that the claim encompasses methods which provide a sample of fetal DNA from cell-free amniotic fluid, wherein the sample comprises both fetal DNA that is cell free and DNA originating from cells in amniotic fluid. The rejection also applies to the claims to the extent the claims encompass methods in which the sample is obtained form amniotic fluid in which the cells have been lysed.

The teachings of Lapierre, and Veltman are presented above. The combined references do not teach applying the prenatal diagnosis method to the detection of a deletion of chromosome 15q11-q13.

However, Lapierre does teach applying array based CGH analysis to the detection of microdeletions observed in Prader-Willi syndrome.

Further, Pinkel teaches methods for detecting chromosomal abnormalities using CGH analysis. Pinkel teaches that the deletion of 15q11-q13 sequences are associated with the occurrence of Prader-Willi syndrome (col. 2, lines 17-21).

In view of the teachings of Lapierre of applying array-based CGH analysis to the detection of deletions associated with Prader-Willi syndrome and the teachings of Pinkel that Prader-Willi syndrome occurs as a result of a deletion of 15q11-q13 sequences, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Lapierre so as to have applied the array-based

CGH analysis to the detection of the deletion of 15q11-q13 chromosomal sequences in order to have provided an effective means of prenatal diagnosis of Prader-Willi syndrome.

Response to Remarks:

In the reply, Applicants state that the claims have been amended to recite using fetal DNA from cell-free amniotic fluid and Lapierre does snot teach using fetal DNA from cell-free amniotic fluid.

This argument is the same as that addressed in full above and applies equally to the present grounds of rejection.

17. **The following are new/modified grounds of rejection necessitated by Applicant's amendments to the claims.** The rejections are applied to the claims to the extent that the claims encompass methods in which fetal DNA is from a sample of amniotic fluid in which substantially all of the cells have been removed from the amniotic fluid prior to the extraction of fetal DNA from the cell-free amniotic fluid, in order to thereby provide a sample of fetal DNA from cell-free amniotic fluid.

18. Claim 27 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lapierre in view of Bianchi (2001) and further in view of Sammons et al (U.S. Patent No. 5,948,278).

Lapierre teaches a method of prenatal diagnosis wherein the sample of amniotic fluid fetal DNA is obtained by removing a first portion of amniotic cells from an amniotic fluid sample to obtain a remaining amniotic material and treating the remaining 5-10 ml of amniotic fluid to extract high molecular weight DNA for CGH analysis (page 124, col.

1). The remaining amniotic fluid thereby contained both amniotic cells and cell free fetal DNA. Lapierre does not teach a method wherein the amniotic fluid sample is obtained by removing substantially all of the cells to obtain cell free DNA.

However, Bianchi teaches a method of prenatal diagnosis comprising obtaining an amniotic fluid sample from pregnant woman at 16-20 weeks of gestation, isolating DNA present in the amniotic fluid sample, performing real-time quantitative PCR using primers and a dual-labeled fluorescent TaqMan probe, and determining the sex of the fetus, thereby providing a prenatal diagnosis (page 1867, col. 2 and page 1868, col. 1). Bianchi teaches that the amniotic fluid fetal DNA is obtained by providing the amniotic fluid sample from a pregnant woman, removing cells from the sample by centrifugation, and treating the remaining amniotic fluid so as to extract cell-free DNA (page 1867, col. 2). The centrifugation step removes substantially all of the cells from the amniotic fluid so that the amniotic fluid fetal DNA consists essentially of cell-free fetal DNA. Further, Bianchi teaches that the amniotic fluid samples are first frozen, stored under appropriate conditions, thawed at 37C prior, and remaining cells are removed by centrifugation prior to treatment to extract DNA (page 1867, col. 2). Bianchi teaches that amniotic fluid contains 100 to 200 fold more fetal DNA per millimeter as compared to maternal plasma (page 1868, col. 1). Bianchi states that “This higher amount may permit new studies and clinical applications to be performed on this typically discarded material” (page 1868, col. 1).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Lapierre so as to have

treated the amniotic fluid as taught by Bianchi and to thereby have performed the prenatal diagnosis method using cell-free fetal DNA because Bianchi teaches that amniotic fluid comprises large quantities of cell-free fetal DNA and thereby this would have provided a highly effective source of fetal DNA for prenatal analysis and a convenient source of fetal DNA since the amniotic fluid supernatant is often available but discarded when the amniotic fluid has been obtained for alternative forms of analysis.

Further, Lapierre teaches obtaining the amniotic fluid sample from woman of "advanced maternal age" but does not specifically teach that the woman are age 35 or older.

Sammons teaches that amniocentesis increases a woman's risk of miscarriage and therefore amniocentesis is generally offered primarily to woman over the age of 35 since woman over the age of 35 have a statistically greater probability of bearing children with congenital defects (col. 1, lines 43-49).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have specifically obtained the amniotic fluid samples from pregnant woman over the age of 35 because these woman would be at a greater risk of having fetuses with a genetic abnormality, and thereby would benefit most from prenatal diagnosis.

19. Claims 68 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lapierre in view of Veltman and Bianchi (2001) and further in view of Sammons et al (U.S. Patent No. 5,948,278).

The teachings of Lapierre, Veltman and Bianchi are presented above. Lapierre teaches obtaining the amniotic fluid sample from woman of "advanced maternal age" but does not specifically teach that the woman are age 35 or older.

Sammons teaches that amniocentesis increases a woman's risk of miscarriage and therefore amniocentesis is generally offered primarily to woman over the age of 35 since woman over the age of 35 have a statistically greater probability of bearing children with congenital defects (col. 1, lines 43-49).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have specifically obtained the amniotic fluid samples from pregnant woman over the age of 35 because these woman would be at a greater risk of having fetuses with a genetic abnormality, and thereby would benefit most from prenatal diagnosis.

20. Claims 77 and 78 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lapierre in view of Veltman, and Bianchi (2001) and further in view of Bianchi (U.S. Patent No. 5,714,325).

The teachings of Lapierre, Veltman and Bianchi (2001) are presented above. The combined references do not teach applying the prenatal diagnosis method to the detection of an X-linked disorder such as Duchenne muscular dystrophy.

However, Bianchi ('325) teaches performing prenatal diagnosis to detect chromosome X deletions associated with Duchenne muscular dystrophy using available probes (col. 14, lines 43-63).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the method of Lapierre in vie w of Veltman to the detection of chromosome X deletions associated with Duchenne muscular dystrophy in order to have provided an effective method of prenatal diagnosis of Duchenne muscular dystrophy.

21. Claim 89 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lapierre in view of Veltman and Bianchi (2001) and further in view of Pinkel (U.S. Patent No. 5,665,549; cited in the IDS).

The teachings of Lapierre, Veltman and Bianchi (2001) are presented above. The combined references do not teach applying the prenatal diagnosis method to the detection of a deletion of chromosome 15q11-q13.

However, Lapierre does teach applying array based CGH analysis to the detection of microdeletions observed in Prader-Willi syndrome.

Further, Pinkel teaches methods for detecting chromosomal abnormalities using CGH analysis. Pinkel teaches that the deletion of 15q11-q13 sequences are associated with the occurrence of Prader-Willi syndrome (col. 2, lines 17-21).

In view of the teachings of Lapierre of applying array-based CGH analysis to the detection of deletions associated with Prader-Willi syndrome and the teachings of Pinkel that Prader-Willi syndrome occurs as a result of a deletion of 15q11-q13 sequences, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Lapierre so as to have applied the array-based CGH analysis to the detection of the deletion of 15q11-q13 chromosomal sequences in

order to have provided an effective means of prenatal diagnosis of Prader-Willi syndrome.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carla Myers whose telephone number is 571-272-0747. The examiner can normally be reached on Monday-Thursday (6:30-5:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dave Nguyen can be reached on 571-272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information

system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Carla Myers/
Primary Examiner, Art Unit 1634